

Detection of Multi-Drug-Resistant extended-spectrum and *AmpC* β -Lactamases-producing *Escherichia coli* from bovine milk samples in West Bengal

L. Sharmila¹, Z. H. Hazarika¹, T. Mondal¹, R. Barua², S. Baidya³, S. Chaudhuri⁴, T. K. Dutta⁵, S. Dey¹,
A. Debnath⁶ and K. Batabyal^{1*}

¹Department of Veterinary Microbiology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, West Bengal, India; ²Department of Veterinary Public Health, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, West Bengal, India; ³Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, West Bengal, India; ⁴Department of Veterinary Medicine, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, West Bengal, India; ⁵Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Aizawl - 796 014, Mizoram, India; ⁶Department of Dairy Technology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, West Bengal, India

Abstract

Escherichia coli is a commensal bacterium that can easily contaminate milk due to unhygienic farm conditions and poor animal health, potentially causing infections in infants and adults. Antimicrobial resistance expressed by *E. coli* in milk can make the disease more fatal. The present study aimed to isolate and characterize 'extended-spectrum' (ESBL) and AmpC (ACBL) β -lactamases producing *E. coli* from milk samples collected from different parts of West Bengal, to assess the potential risk associated with the food. A total of 107 bovine milk samples were collected from apparently healthy cows to isolate *E. coli*. The ESBL and ACBL-positive *E. coli* strains were detected both phenotypically and genotypically. An antibiogram of the ESBL-positive isolates was also tested. In this study, 51 (47.7%) milk samples were found to be positive for *E. coli*, confirmed by the detection of the *16S rRNA* gene specific to the genus. Six (11.7%) *E. coli* strains were positive for phenotypic ESBL production and 28 (54.9%) isolates were ACBL producers *in vitro*. The identified β -lactamase genes in isolates were *bla*_{CTX-M} (8, 15.7%), *bla*_{SHV} (4, 7.8%) and 36 (70.5%) *bla*_{AmpC} genes, but no *bla*_{TEM} gene. Three (5.88%) isolates were positive for CTX-M and AmpC genes. Antibiogram of these ESBL and ACBL positive (n=48) isolates revealed that drugs like co-trimoxazole (83.33%), chloramphenicol (81.25%), erythromycin (60.42%) and streptomycin (64.58%) were sensitive against these pathogens but drugs *viz.* ampicillin (100%), amoxicillin/clavulanic acid (70.83%), oxytetracycline (60.42%), cefalexin (93.75%), cefotaxime (87.5%), cefadroxil (72.91%), were resistant.

Keywords: ACBL, Antibiogram, Bovine milk, ESBL, *Escherichia coli*

Highlights

- Around 47.7% milk samples tested in this study were found to be positive for *E. coli*, among which 11.7% were positive for ESBL production genotypically.
- Genotypic ACBL positivity was detected in 70.5% of isolates in this study.
- The β -lactamases-producing *E. coli* isolates were detected to be resistant to a few commonly used antibiotics, whereas antimicrobials, *viz.*, co-trimoxazole, erythromycin, chloramphenicol, and streptomycin, were effective against these strains.

INTRODUCTION

In India, a vast majority (almost 80%) of milk production relies on small dairy farmers and is sold through the unorganized sector. While this system offers economic and social benefits, it also raises food safety concerns. These unorganized farms often lack the necessary resources, infrastructure and access to animal health services. Milk is a significant pathway

for foodborne pathogens to spread from animals to humans. Furthermore, consuming milk tainted with antimicrobial residues or resistant bacteria poses a significant risk for the proliferation of antimicrobial resistance (AMR). *Escherichia coli*, a Gram-negative bacillus under the family *Enterobacteriaceae*, can cause serious food-borne infections in human beings, affecting the consumer's health. The pathotype of

*Corresponding Author, E-mail: drkb.micro@gmail.com

E. coli, i.e., 'enterohemorrhagic *E. coli* (EHEC)' can be more dangerous with a great adverse effect on human health (Detzner et al., 2022).

The increasing incidence of AMR among the pathogenic strains is directly related to the increase in ESBL and ACBL-producing pathogens all over the globe. Gram-negative bacteria like *E. coli* possess extended β -lactamases (ESBL) and AmpC (ACBL) β -lactamases, which can nullify the action of many antibiotics, even the 2nd or 3rd generation antibiotics commonly used for treating infections (Husna et al., 2023). *E. coli* strains carrying the resistance genes can easily transfer those genes to other pathogens, leading to the spread of drug resistance (Detzner et al., 2022). These pathogens pose a major challenge to the treatment of even general infections and cause serious human health hazards. So, the presence of β -lactamases-producing *E. coli* in the food processing chain or in the food of our daily consumption, which is possibly coming from these healthy/unhealthy farm animals, may be a matter of great concern (Ling et al., 2021). In this background, the present study aimed to detect and characterize ESBL and ACBL-producing *E. coli* strains from raw cattle milk samples collected from different districts of West Bengal, followed by further characterization and to determine their antibiotic resistance patterns *in vitro*.

MATERIALS AND METHODS

Milk sample collection: From April to June, 2023, a total of 107 milk samples were aseptically collected from rural dairy farmers of different districts of West Bengal, namely Birbhum, 24 PGs south and north, Hooghly, and Nadia. Ten (10) mL of random milk was collected directly from the teats of the animals into sterile containers. The samples under cold chain were transported to the Department of Veterinary Microbiology, WBUAFS, Kolkata. All the samples were analyzed on the same day of receipt at the lab.

Isolation and characterization of *Escherichia coli*:

All the milk samples were enriched with sterile nutrient broth (Hi-Media, India) at 37°C for 16-18 hours, followed by streaking onto MacConkey's agar (Hi-Media, India) plates. The plates were incubated aerobically at 37°C for 24 hours. All tentative pinkish single colonies (i.e., lactose fermenting) were selected for selective isolation by further streaking on Eosin Methylene Blue (EMB) agar (Hi-Media, India) plates, by incubating for 24 hours at 37°C (Samanta, 2013). The colonies showing characteristic greenish 'metallic sheen' on EMB agar were picked up and

stored using sterile nutrient agar slants for further study.

Morphological characterization done by Gram's staining, and biochemical characterization with tests like indole, methyl red, citrate utilization, and Voges-Proskauer [IMViC] (Samanta, 2013). All positive isolates were typically identified as *Escherichia coli* and were further confirmed by PCR.

Confirmation of the *Escherichia coli* isolates: All positive *E. coli* isolates were confirmed by PCR detection of the *16S rRNA* gene (585bp) specific for the genus *Escherichia* (Amit-Romach et al., 2004). Details of the primers used, and the PCR protocol and conditions are narrated in Table 1.

Phenotypic detection of ESBL and ACBL production in *E. coli* isolates: The phenotypic detection of Extended-Spectrum β -Lactamase (ESBL) in *E. coli* isolates was performed *in vitro* using the disc diffusion method, adhering to Clinical and Laboratory Standards Institute [CLSI] (2020) guidelines. This involved using both Cefotaxime (30 μ g) and ceftazidime (30 μ g) disks, with and without clavulanate (10 μ g). ESBL production was phenotypically confirmed if the zone diameter difference between a cefotaxime, ceftazidime and its corresponding clavulanate disk was greater than 5 mm.

Additionally, all *E. coli* isolates were subjected to the ceftazidime-clavulanate double disc synergy (CC-DDS) test for phenotypic confirmation of AmpC β -lactamase production (Shaaban et al., 2022).

Molecular detection of ESBL and ACBL-producing genes in *E. coli* isolates by PCR

Bacterial culture lysate preparation: Selective *E. coli* strains were inoculated into the nutrient broth (Hi-Media, India), followed by 18-hour incubation at 37°C. One mL of young broth culture of each sample was taken in a sterile 1.5 mL micro-centrifuge tube (Tarsons, India) followed by centrifugation at 6000 rpm for 5 minutes. The obtained pellet was washed thrice with TE buffer and suspended again in TE buffer (1 mL). The micro-centrifuge tube with culture was then boiled in water for 10 minutes, then immediately chilled on ice to lyse the cells, denature proteins, and release DNA, relying on heat to break down cellular structures and cold to stop enzymes like DNases, making it suitable for simple PCR/amplification tests but yielding less pure DNA than kits. Then, each tube was centrifuged at 5000 rpm for 5 minutes, followed by the removal of cell debris, and the supernatant with crude DNA was collected and stored at -20°C for further use as a template in PCR (Osman & Waheed, 2017).

Detection of the bla_{CTX-M} gene in *E. coli* isolates: All phenotypically ESBL-positive *E. coli* isolates were considered for confirmation by PCR detection of the bla_{CTX-M} gene in them as per the protocol of Weill et al. (2004) with slight modifications by adjusting the annealing temperature. All standard reagents and primers (GCC Biotech, India) were used in this process (Table 1). The amplified PCR products were loaded onto a 1.5% w/v agarose gel (SRL, India), with ethidium bromide (0.5 µg/mL) (SRL, India), followed by agarose gel electrophoresis, and were visualized by gel documentation system (UVP, UK). For all PCR assay a positive control was provided by a departmental isolate of *E. coli*, which had been previously confirmed as an ESBL- and ACBL-producing strain. A blank was maintained as the negative control.

Detection of the bla_{TEM} gene in *Escherichia coli* isolates by PCR amplification: The bla_{TEM} genes in *E. coli* isolates were detected by PCR assay as per Farzi et al. (2021), with some modifications by increasing the extension time for the long target gene. The primer sequences and detailed PCR conditions are listed in Table 1. The PCR products were visualized after gel electrophoresis by a gel documentation system (UVP, UK).

Detection of the bla_{SHV} gene of *E. coli* isolates by PCR amplification: For the detection of the bla_{SHV} genes in *E. coli* isolates, PCR was performed as described by Cao et al. (2002) with some modifications (by lowering the denaturation temperature by 1°C). The primer sequences and detailed PCR conditions are listed in Table 1. The products were visualized after electrophoresis using a gel documentation system (UVP, UK) and photographed.

Detection of the bla_{AmpC} gene of *E. coli* isolates by PCR amplification: For the detection of the bla_{AmpC} genes in *E. coli* isolates, PCR was performed as per the protocol described by Féria et al. (2002) with some modifications by increasing the time of annealing. The primer sequences and detailed PCR conditions are listed in Table 1. The PCR products were electrophoresed and visualized in a gel documentation system (UVP, UK).

In-vitro Antibiotic Sensitivity Test (AST) of ESBL and ACBL-positive *E. coli* isolates: Antibigram of all the ESBL+ACBL-producing *E. coli* isolates was performed using 12 antimicrobials, i.e. ampicillin, amoxicillin/clavulanic acid, tetracycline, erythromycin, streptomycin, oxytetracycline, cefalexin, cefotaxime, cefadroxil, cefixime, co-trimoxazole, and chloramphenicol by Kirby-Bauer Disc diffusion method (CLSI, 2020). Fresh broth cultures of all the selected *E. coli* isolates were produced for the test. Separate and sterile Mueller-Hinton agar (Hi-Media, India) plates were used to spread each broth culture (with 0.5 McFarland turbidity, equivalent to 1.5×10^8 bacteria per mL) using a sterile L-spreader, and standard discs (Hi-Media, India) were placed with sterile forceps. All the plates were incubated at 37°C for 24 hours, and the results were interpreted by measuring the zone of inhibition diameter and comparing it with the standard chart provided by the company.

RESULTS

Isolation, characterization, and confirmation of the *Escherichia coli* isolates: Out of 107 bovine milk samples, 51(47.66%) samples were positive for *Escherichia coli* (Table 1). All the positive isolates

Table 1. Details of Primers used and PCR conditions followed in this study

Target gene	Reaction Mixtures (25µL)	PCR Conditions
<i>E. coli</i> 16S rRNA (585bp) [Amit-Romach et al., 2004]	F: GACCTCGGTTTAGTTACAGAR: CAC ACGCTGACGCTGACCA5 µL of extracted <i>E. coli</i> DNA samples which were added to 20 µL of PCR mixture containing 2 mM MgCl ₂ , 0.2 mM of each deoxynucleoside triphosphate, 0.25 mM of each primer, and 1U of Taq DNA polymerase	Initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 1 min, elongation at 72°C for 1 min, and final extension at 72°C for 10 min.
bla_{TEM} gene (867bp)[Farzi et al., 2021]	F: ATGAGTATTCAACATTTCCGR: CGTA CAGTTACCAATGCTTA5 µL of DNA templates, 20 picomol of each primer, 2.5 mM MgCl ₂ , 0.2 mM of each dNTPs, 1U Taq polymerase and 5x Taq buffer (Promega, USA)	Initially at 95°C for 5 mins, followed by 35 cycles of 1 min at 95°C (denaturation), 1min at 55°C (annealing) and 1 min at 72°C (extension) with the final extension at 72°C for 10 min.

Cont. Table 1.

Table 1., Cont. ...

Target gene	Reaction Mixtures (25µL)	PCR Conditions
<i>bla</i> _{SHV} gene (792bp)[Cao et al., 2002]	F: TTATCTCCCTGTAGCCACCR: GATT TGCTGATTTCGCTCGG5 µL DNA templates, 2 mM MgCl ₂ , 200 iM dNTPs, 50 picomoles of each primer, 1U Taq DNA polymerase (Promega, USA), and 10% dimethyl sulfoxide (DMSO)	Initial denaturation of 10 minutes at 94°C, followed by denaturation at 94°C for 45 seconds, annealing at 52°C for 1 min, extension at 72°C for 1 min for 35 cycles, and final extension at 72°C for 10 min.
<i>bla</i> _{CTX-M} gene (540bp)[Weill et al., 2004]	F: CAATGTGCAGCACCAAGTAAR: CGC GATATATCGTTGGTGGTGGTGFive µL DNA templates, 50 pmol of each primer, 200 mM dNTPs, 1U Taq DNA polymerase (Promega, USA), 2 mM MgCl ₂ , and 10% dimethyl sulfoxide	Initial denaturation at 94°C followed by 30s of denaturation at 94°C (10 min), 30s of annealing at 53°C, 1 min of extension at 72°C for 35 cycles, and 10 min of final extension at 72°C
<i>bla</i> _{AmpC} gene (634bp)[Féria et al., 2002]	F: CCCCCTTATAGAGCAACAAR: TCA ATGGTCGACTTCACACC1U of Taq DNA Polymerase (Promega, USA), 2 mM MgCl ₂ , 200 mM of each dNTPs, 100 pmol of each primer, 10% dimethyl sulfoxide & 5 µL of DNA templates	The initial denaturation step of 5 min at 94°C; followed by 30 cycles of amplification consisting of 30s of denaturation at 94°C, 30s of annealing at 57°C, 1 min of elongation at 72°C and 10 min of final extension at 72°C

showed typical 'metallic sheen' on EMB agar plates. Morphological examination after Gram's staining revealed them to be Gram-negative bacilli. Further biochemical characterization provided typical results, *i.e.* positive

to indole and methyl red, whereas negative to VP and citrate utilization tests. Confirmation of these isolates was achieved by detecting *16S rRNA* genes specific to the *Escherichia coli* genus.

Detection of ESBL and ACBL activity in *E. coli* isolates phenotypically: Phenotypic analysis revealed that 6 (11.76%) of the *E. coli* isolates produced ESBL, whereas 28 (54.90%) isolates were positive for ACBL production *in vitro* (Table 2).

***In-vitro* Antibiotic Sensitivity Test (AST) of ESBL and ACBL-positive *E. coli* isolates:** *In vitro*, antibiotic sensitivity assay of the ESBL and ACBL-positive *E. coli* isolates showed high-level resistance to ampicillin, cefotaxime, cefixime, amoxicillin-clavulanic acid, tetracycline, cefadroxil, and

Table 2. District-wise detection of *E. coli* strains with phenotypical beta-lactamase production

Name of the Districts	No. of milk samples screened	No. of <i>E. coli</i> strains isolated (%)	Phenotypically ESBL+ACBL Positive Isolates	<i>bla</i> _{CTXM}	<i>bla</i> _{SHV}	<i>bla</i> _{AmpC}
Birbhum	14	5	1+1	2	1	2
24 PGs (S)	24	14	2+7	2	0	5
24 PGs (N)	11	4	0+3	1	0	4
Hooghly	27	12	2+6	2	2	11
Nadia	31	16	1+11	1	1	14
Total	107	51 (47.66%)	34 (66.67%)	8 (15.67%)	4(7.84%)	36(70.59%)

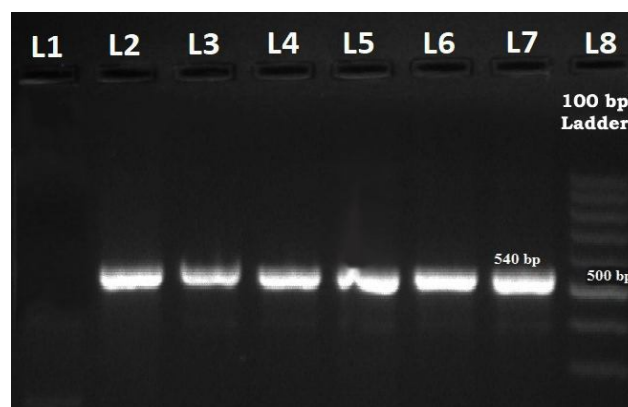


Fig. 1. PCR detection of the *bla*_{CTX-M} gene (540bp) in ESBL-producing *E. coli* strains isolated from bovine milk samples in West Bengal (Lane 1: Uninoculated negative control, L2: Positive control, L3-L7: Test samples, L8: 100 bp ladder)

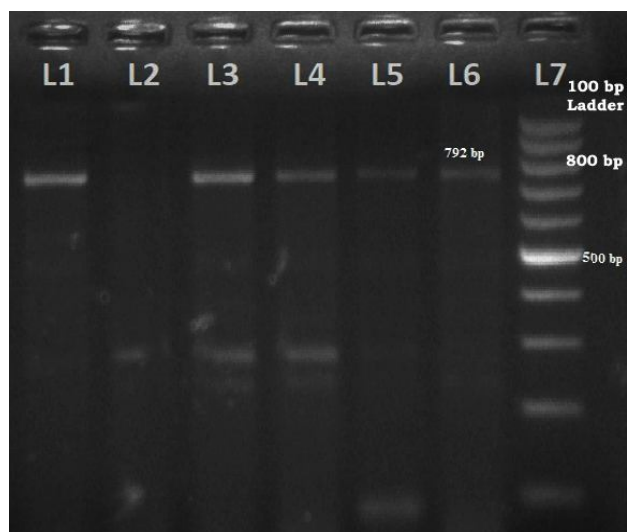


Fig. 2. PCR detection of the *bla*_{SHV} gene (792bp) in ESBL-producing *E. coli* strains isolated from bovine milk samples (Lane 1: Positive control, L2: Uninoculated Negative control, L3-L6: Test samples, L7: 100bp Ladder)



Fig. 3. PCR detection of the *bla*_{AmpC} gene (634bp) in ACBL-producing *E. coli* strains isolated from bovine milk samples in West Bengal (Lane 1: 100 bp ladder, L2-L6: Test samples, L7: Positive control, Lane 8: Uninoculated negative control)

Table 3. *In vitro* resistance patterns of β -lactamases producing *E. coli* strains (n=48) against commonly used antimicrobials

Sl. No.	Antimicrobials (Conc. in μ g)	Isolates sensitive		Intermediately sensitive		Isolates resistant	
		No.	%	No.	%	No.	%
1.	Ampicillin	0	0	0	0	48	100
2.	Amoxicillin / Clavulanic acid	0	0	14	29.17	34	70.83
3.	Tetracycline	0	0	5	10.42	43	89.58
4.	Erythromycin	29	60.42	0	0	19	39.58
5.	Streptomycin	31	64.58	1	2.08	16	33.33
6.	Oxytetracycline	14	29.17	5	10.42	29	60.42
7.	Cefalexin	3	6.25	0	0	45	93.75
8.	Cefotaxime	6	12.50	0	0	42	87.50
9.	Cefadroxil	11	22.92	2	4.17	35	72.91
10.	Cefixime	17	35.42	3	6.25	20	58.33
11.	Co-Trimoxazole	40	83.33	2	4.17	6	12.50
12.	Chloramphenicol	39	81.25	4	8.33	5	10.42

oxytetracycline, etc. (with the range of 60-100%). Very few drugs like erythromycin, streptomycin, co-trimoxazole, and chloramphenicol (60-83%) were sensitive to these pathogens (Table 3).

DISCUSSION

Fifty-one (47.66%) samples tested here were detected to be positive, yielding *Escherichia coli*, which was also supported by Fahim et al. (2019), and Megersa et al. (2019), who reported 42-50% *E. coli* positivity in cow milk. Again, earlier reports by Dogan et al. (2012), and Ali et al. (2016) showed 12-13% *E. coli* positivity in the bovine milk samples which is

quite lower than the current report. The typical cultural, morphological, and biochemical characterization of these isolates was supported by Samanta (2013), and Mondal et al. (2024). The PCR confirmation of the presence of the *16S rRNA* gene was supported by Amit-Romach et al. (2004) and Banerjee et al. (2022). In the present study, 11.76% of the *E. coli* isolates were phenotypically positive for ESBL production, whereas 54.9% of the isolates were ABCL positive, which was supported by Kazemian et al. (2019). Salvia et al. (2022) isolated 50.4% EBSL positivity in *E. coli*, which is higher than the present report. El-Hariri et al. (2023) detected 48 (39.67%) ESBL-positive

E. coli isolates in their study, which almost matches the current findings.

Eight (8) *E. coli* isolates were positive for the *bla*_{CTX-M} gene, 4 isolates were positive for the *bla*_{SHV}, and 36 (70.59%) isolates for the *bla*_{AmpC} gene as found in this study. The frequency of the AmpC gene was the highest, followed by CTX-M and SHV (9.8%). Na et al. (2019) reported six (4.1%) ESBL-positive *E. coli*, carrying *bla*_{CTX-M} genes from 146 *E. coli* isolates in their study. El-Hariri et al. (2023) found the ESBL-positive *E. coli* isolates with the *bla*_{TEM} (25%), to be the most prevalent gene, followed by *bla*_{CTX-M} (19.08%) and *bla*_{SHV} (16.45%), which partially matches the current findings. Sadeghi et al. (2022) reported *E. coli* isolated from UTI infections as ESBL producers, and *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} were detected in 42 (34.7%), 44 (36.4%), and 47 (38.8%) cases, respectively, which also supports the current findings. Such frequent presence of antibiotic-resistant genes in *E. coli* may be of great concern as these may also be passed to human consumers as well as calves, leading to the spread of antibiotic resistance. *In vitro*, antibiotic sensitivity assay of the ESBL and ACBL-positive *E. coli* isolates showed high-level resistance that were also reported earlier by Sadeghi et al. (2022), and El-Hariri et al. (2023) in their studies. All the ESBL-producing *E. coli* isolates were resistant to ampicillin (100%) and approximately 61-86% resistant to norfloxacin, ertapenem, ciprofloxacin, tetracycline, and ceftazidime, respectively, as reported by Mondal et al. (2022), which is quite similar to the present findings. ESBL-producing *E. coli* isolated from pet animals showed phenotypical resistance to tetracycline (93.1%), azithromycin (89.8%), ampicillin (84.2%), cefotaxime (80.9%), doxycycline (82.5%), co-trimoxazole (80.9%), and ampicillin/cloxacillin (76.9%), which are in agreement with the present study was reported by Banerjee et al. (2022)

It can be concluded that around 48% of milk samples were found to be infected with antibiotic-resistant *E. coli* isolates. ESBL (15.7%) and ACBL genes (70.5%) are significantly present in these isolates, which may be of great health concern for human beings. These isolates were highly resistant to several commonly used antibiotics. As per this report, proper care should be taken to prevent foodborne illness from these pathogens.

REFERENCES

Ali, T., Ur Rahman, S., Zhang, L., Shahid, M., Zhang, S., Liu, G., Gao, J., & Han, B. (2016). ESBL-Producing *Escherichia coli* from cows suffering mastitis in China contain clinical Class 1 Integrons with CTX-M

Conflict of interest: There is no competing interest between the authors of this article.

Author's contribution: LS, ZHH: Involved in investigation and data generation; TM: Prepared the original draft; RB, SB, SC, AD: Engaged in conceptualization, data processing, analysis, supervision, and final editing; TKD: Arranged all the funds for the work; SD, KB: Involved in data analyses, methodology finalization and final editing.

Data availability statement: The data set generated during research will be available from the corresponding author upon reasonable request.

Ethical approval: As there is no use of any laboratory or domestic animals for experimental research, no institutional animal ethics committee clearance is required for this study. But the research work got approval from the institutional biosafety committee for handling bacterial pathogens as per standard methods in compliance with national guidelines.

Use of artificial intelligence tools: During the preparation of this work, the author(s) used no artificial intelligence tools, so no further declaration is required in this regard.

ACKNOWLEDGMENT

The authors thank the University Authorities and the Faculties of the Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, West Bengal, India, for providing the necessary research facilities for this study. Necessary fund support in terms of consumables and chemicals by the research scheme of the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Aizawl entitled "Establishment of Consortium for One Health to address Zoonotic and trans-boundary diseases in India, including the Northeast India" funded by the Department of Biotechnology, Govt. of India (Order no. BT/PR39032/ADV/90/285/2020) is duly acknowledged. They are also thankful to the farm owners, dairy farmers, and local veterinary doctors of the concerned districts of West Bengal, India, for their technical and field support.

linked to ISCR1. *Frontiers in Microbiology*, 7, 1931. <https://doi.org/10.3389/fmicb.2016.01931>
Amit-Romach, E., Sklan, D., & Uni, Z. (2004). Microflora ecology of the chicken intestine using 16S ribosomal DNA

- primers. *Poultry Science*, 83(7), 1093-1098. <https://doi.org/10.1093/ps/83.7.1093>
- Banerjee, A., Pal, S., Goswami, P., Batabyal, K., Joardar, S. N., Dey, S., Isore, D. P., Dutta, T. K., Bandyopadhyay, S. K., & Samanta, I. (2022). Docking analysis of circulating CTX-M variants in multi-drug resistant, beta-lactamase and biofilm-producing *E. coli* isolated from pet animals and backyard livestock. *Microbial Pathogenesis*, 170, 105700. <https://doi.org/10.1016/j.micpath.2022.105700>
- Cao, V., Lambert, T., Nhu, D. Q., Loan, H. K., Hoang, N. K., Arlet, G., & Courvalin, P. (2002). Distribution of extended-spectrum β -lactamases in clinical isolates of *Enterobacteriaceae* in Vietnam. *Antimicrobial Agents and Chemotherapy*, 46(12), 3739-3743. <https://doi.org/10.1128/aac.46.12.3739-3743.2002>
- Clinical and Laboratory Standards Institute. (2020). *Performance Standards for Antimicrobial Susceptibility Testing: Supplement M100* (30th ed.). Clinical and Laboratory Standards Institute.
- Detzner, J., Pohlentz, G., & Muthing, J. (2022). Enterohemorrhagic *Escherichia coli* and a fresh view on shiga toxin-binding glycosphingolipids of primary human kidney and colon epithelial cells and their toxin susceptibility. *International Journal of Molecular Sciences*, 23(13), 6884. <https://doi.org/10.3390/ijms23136884>
- Dogan, B., Rishniw, M., Bruant, G., Harel, J., Schukken, Y. H., & Simpson, K. W. (2012). Phylogroup and *lpfA* influence epithelial invasion by mastitis associated *Escherichia coli*. *Veterinary Microbiology*, 159(1-2), 163-170. <https://doi.org/10.1016/j.vetmic.2012.03.033>
- El-Hariri, S. A., Saleh, F., Moghnieh, W., & Sokhn, E. S. (2023). Phenotypic and molecular characterization of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* among Lebanese patients. *JAC-Antimicrobial Resistance*, 5(3), dlad074. <https://doi.org/10.1093/jacamr/dlad074>
- Fahim, K. M., Ismael, E., Khalefa, H. S., Farag, H. S., & Hamza, D. A. (2019). Isolation and characterization of *E. coli* strains causing intramammary infections from dairy animals and wild birds. *International Journal of Veterinary Science and Medicine*, 7(1), 61-70. <https://doi.org/10.1080/23144599.2019.1691378>
- Farzi, S., Ranjbar, R., Niakan, M., & Ahmadi, M. H. (2021). Molecular characterization of antibiotic resistance associated with TEM and CTX-M ESBL in uropathogenic *E. coli* strains isolated from outpatients. *Iranian Journal of Pathology*, 16(4), 386-391.
- Féria, C., Ferreira, E., Correia, J. D., Gonçalves, J., & Caniça, M. (2002). Patterns and mechanisms of resistance to β -lactams and β -lactamase inhibitors in uropathogenic *Escherichia coli* isolated from dogs in Portugal. *Journal of Antimicrobial Chemotherapy*, 49(1), 77-85. <https://doi.org/10.1093/jac/49.1.77>
- Husna, A., Rahman, M. M., Badruzzaman, A. T. M., Sikder, M. H., Islam, M. R., Rahman, M. T., Alam, J., & Ashour, H. M. (2023). Extended-Spectrum β -Lactamases (ESBL): challenges and opportunities. *Biomedicines*, 11(11), 2937. <https://doi.org/10.3390/biomedicines11112937>
- Kazemian, H., Heidari, H., Ghanavati, R., Ghafourian, S., Yazdani, F., Sadeghifard, N., Valadbeigi, H., Maleki, A., & Pakzad, I. (2019). Phenotypic and genotypic characterization of ESBL-, AmpC-, and carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates. *Medical Principles and Practice*, 28(6), 547-551. <https://doi.org/10.1159/000500311>
- Ling, W., Furuya-Kanamori, L., Ezure, Y., Harris, P. N. A., & Paterson, D. L. (2021). Adverse clinical outcomes associated with infections by Enterobacterales producing ESBL (ESBL-E): a systematic review and meta-analysis. *JAC-Antimicrobial Resistance*, 3(2), dlab068. <https://doi.org/10.1093/jacamr/dlab068>
- Megersa, R., Mathewos, M., & Fesseha, H. (2019). Isolation and identification of *Escherichia coli* from dairy cow raw milk in Bishoftu town, Central Ethiopia. *Archives of Veterinary and Animal Sciences*, 1(1), 1-7
- Mondal, T., Batabyal, K., Dey, S., Joardar, S. N., Samanta, I., Isore, D. P., Samanta, S., & Paul, A. (2022). Detection and characterization of β -lactamases and biofilm-producing *Escherichia coli* and *Klebsiella pneumoniae* from ducks and duck environments in West Bengal. *Indian Journal of Animal Research*, 58(7): 1196-1202. <https://doi.org/10.18805/ijar.b-4915>
- Mondal, T., Samanta, S., Dey, S., Samanta, I., Joardar, S. N., Abhiram, S., Paul, A., Bardhan, R., Bhowmick, S., & Batabyal, K. (2024). Detection of antimicrobial resistance in salmonella enterica isolated from ducks and their environments in West Bengal, India. *Exploratory Animal and Medical Research*, 14 (Superbug Spl.), 96-104. [https://doi.org/10.52635/eamr/14\(s2\)96-104](https://doi.org/10.52635/eamr/14(s2)96-104)
- Na, S. H., Moon, D. C., Choi, M., Oh, S., Jung, D., Sung, E. J., Kang, H. Y., Hyun, B., & Lim, S. (2019). Antimicrobial resistance and molecular characterization of extended-spectrum β -lactamase-producing *Escherichia coli* isolated from ducks in South Korea. *Foodborne Pathogens and Disease*, 16(12), 799-806. <https://doi.org/10.1089/fpd.2019.2644>
- Osman, N., & Waheed, D. (2017). Virulence associated genes and antibiotic resistance profiles in *Salmonella* species isolated from chickens. *International Journal of Poultry Science*, 16(8), 303-309. <https://doi.org/10.3923/ijps.2017.303.309>
- Sadeghi, M., Ebrahim-Saraie, H. S., & Mojtahedi, A. (2021). Prevalence of ESBL and AmpC genes in *E. coli* isolates from urinary tract infections in the north of Iran. *New Microbes and New Infections*, 45, 100947. <https://doi.org/10.1016/j.nmni.2021.100947>
- Salvia, T., Dolma, K. G., Dhakal, O. P., Khandelwal, B., & Singh, L. S. (2022). Phenotypic detection of ESBL, AmpC, MBL, and their co-occurrence among MDR *Enterobacteriaceae* isolates. *Journal of Laboratory Physicians*, 14(03), 329-335. <https://doi.org/10.1055/s-0042-1744239>
- Samanta, I. (2013). Chapter 6: *Enterobacteriaceae* (pp. 119-135). In *Veterinary Bacteriology*. New India Publishing Agency.
- Shaaban, M., Elshaer, S. L., & Abd El-Rahman, O. A. (2022). Prevalence of extended-spectrum β -lactamases, AmpC, and carbapenemases in *Proteus mirabilis* clinical isolates. *BMC Microbiology*, 22(1), 247. <https://doi.org/10.1186/s12866-022-02662-3>
- Weill, F. X., Perrier-Gros-Claude, J. D., Demartin, M., Coignard, S., & Grimont, P. A. (2004). Characterization of extended-spectrum- β -lactamase (CTX-M-15)-producing strains of *Salmonella enterica* isolated in France and Senegal. *FEMS Microbiology Letters*, 238(2), 353-358. <https://doi.org/10.1111/j.1574-6968.2004.tb09776.x>

Received - 03.06.2025, Accepted - 12.01.2026, Published - 23.03.2026 (Online), 01.06.2026 (Print)

Section Editor: Dr. Neeraj Shrivastava, Editorial Board Member